SUPPLEMENTAL MATERIAL

Association between Lead and Cadmium and Reproductive Hormones in Peripubertal U.S. Girls

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Supplemental Material, Technical Note 1

Laboratory Methods

Inhibin B was measured using the DSL-10-84100 ACTIVE® Inhibin B ELISA assay (Gen I assay), an enzymatically amplified two-site two-step sandwich-type immunoassay and concentration was calculated from the standard curve. For inhibin B, the intra-assay coefficients of variation (CV) were 3.5%, 4.6% and 5.6% for means correlated with 69, 274, and 472 pg/mL, respectively, while the inter-assay CVs were 7.6%, 6.3% and 6.2% for means of 50.1, 188.4, and 355.0 pg/ml, respectively. Since the inhibin B analyses were run, a Generation II assay (Gen II) for inhibin B has been introduced that yields levels more comparable with other commercially available assays (Kalra et al. 2009; Ludlow et al. 2008). Given the strong correlation ($r^2 = 0.94$) between the Gen I and Gen II assays, the Gen I results are comparable across studies using the following equation: Gen II = 1.57(Gen I) + 11.29 pg/mL (Kalra et al. 2009).

LH was measured using the LH ELISA kit (Bio-Quant BQ049F), a solid phase direct sandwich method. For luteinizing hormone (LH), the intra-assay CVs were 10.6%, 7.6% and 6.2% for means of 1.7, 16.3, and 47.2 mIU/mL, respectively, and inter-assay CVs were 11.6%, 10.8% and 8.1% for means of 1.9, 15.6, and 46.1 mIU/mL, respectively.

Supplemental Material, Technical Note 2

Comment on Lead and Growth

Our finding that girls who exceeded the inhibin B pubertal cutoff were taller is consistent with previous studies in NHANES III that showed height is positively associated with Tanner stage and maturational process (Selevan et al. 2003). However, we also observed that girls above the inhibin B cutoff were relatively thinner, on average, than those below the cutoff in terms of lower body mass index (BMI) after age adjustment, which differs from the findings of others using NHANES III pubertal staging data among girls aged 8-18 years (Selevan et al. 2003; Wu et al. 2003). This contrast may be explained, in part, by the fact that our sample was younger than those in previous studies using NHANES III (6-11 years versus 8-18 years). Our finding extended to younger ages may indicate differences in the longitudinal progression of peripubertal growth from an initial increase in height to a later increase in body mass and fatness. The most dramatic increase in body mass occurs at later pubertal stages (Guo et al. 1998) and may not have been fully observed in a sample of this age range and developmental stage (80% Tanner stage ≤ 2). Additional adjustment for height did not substantially alter the association between Pb and hormones. In addition, we observed that non-Hispanic black girls had significantly higher levels of inhibin B than non-Hispanic white girls, consistent with earlier reports that non-Hispanic black girls enter puberty earlier than non-Hispanic white girls (Wu et al. 2002). We found racial/ethnic differences in blood lead levels with non-Hispanic black girls having the highest lead levels followed by Mexican-Americans and non-Hispanic whites supporting earlier reports (Ballew et al. 1999). However, even within the non-Hispanic black racial/ethnic group, girls with higher Pb levels had lower inhibin B concentrations than girls with lower Pb levels ($p = 0.03$).

Supplemental Material, Technical Note References

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Supplemental Material, Table 1

Association of blood lead with inhibin B and LH concentrations using a quantile analysis

Blood Lead Concentration	n	Unadjusted OR	95% CI	Multivariable-Adjusted[a] OR	95% CI
Relative prevalence of Inhibin B ≥80th percentile versus <LOD					
Continuous Lead					
Log-lead	522	0.62	(0.43, 0.88)	0.72	(0.55, 0.94)
Categorical Lead					
Low:					
< 1 µg/dL	53	Reference		Reference	
Moderate:					
1-4.9 µg/dL	370	0.61	(0.33, 1.28)	0.51	(0.23, 1.12)
High:					
≥5 µg/dL	99	0.37	(0.17, 0.80)	0.31	(0.12, 0.83)
Relative prevalence of Inhibin B 60-79th percentile versus <LOD					
Continuous Lead					
Log-lead	522	0.90	(0.67, 1.22)	0.91	(0.70, 1.19)
Categorical Lead					
Low:					
< 1 µg/dL	50	Reference		Reference	
Moderate:					
1-4.9 µg/dL	364	0.68	(0.36, 1.28)	0.71	(0.35, 1.43)
High:					
≥5 µg/dL	108	0.68	(0.32, 1.40)	0.70	(0.30, 1.60)

Relative prevalence of LH ≥75th percentile versus <LOD			
Continuous Lead			
Log-lead	489	0.81 (0.63, 1.03)	0.69 (0.49, 0.96)
Categorical Lead			
Low:			
< 1 µg/dL	56	Reference	Reference
Moderate:			
1-4.9 µg/dL	342	0.91 (0.43, 1.97)	1.01 (0.56, 2.34)
High:			
≥5 µg/dL	91	0.58 (0.28, 1.18)	0.48 (0.19, 1.22)
Relative prevalence of LH 49th-74th percentile versus <LOD			
Continuous Lead			
Log-lead	502	1.08 (0.85, 1.36)	0.95 (0.73, 1.24)
Categorical Lead			
Low:			
< 1 µg/dL	52	Reference	Reference
Moderate:			
1-4.9 µg/dL	346	1.19 (0.61, 2.27)	1.31 (0.70, 2.44)
High:			
≥5 µg/dL	104	0.84 (0.39, 1.80)	1.14 (0.56, 2.34)

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a = Adjusted for age (months), race/ethnicity (non-Hispanic white as referent), body mass index (kg/m^2), poverty/income ratio and Census region (Northeast as referent)

LH, luteinizing hormone; OR, adjusted odds ratio; CI, confidence interval